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Prevalence of GBV-C/Hepatitis G Virus RNA and E2 Antibody Among Subjects Infected With Human Immunodeficiency Virus Type 1 After Parenteral or Sexual Exposure

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GB virus C (GBV-C) or hepatitis G virus (HGV) is transmitted by the parenteral route but the importance of sexual transmission needs to be ascertained. GBV-C/HGV infections were investigated using RNA and E2-antibody detection methods in 80 subjects infected by the human immunodeficiency virus type 1 (HIV-1) divided into 4 groups of 20 individuals each according to their main risk factor for HIV-1 infection: blood product recipients (group 1), intravenous drug users (group 2), homosexuals (group 3), or heterosexual exposure (group 4). The overall prevalence of GBV-C/HGV infection was 66.3%. No significant difference was observed in GBV-C/HGV prevalence among the four groups: 75, 75, 55, and 60% in groups 1, 2, 3, and 4, respectively. Hepatitis C virus (HCV) antibodies, used as a control for parenteral exposure, were found in 70% and 90% of the subjects in groups 1 and 2 versus only 15% and 20% of the subjects in groups 3 and 4, respectively ($P < .001$). Similarly, coinfections with GBV-C/HGV and HCV were significantly associated with the parenteral route ($P < .001$). These data emphasized the usefulness of combining the detection of RNA and the E2 antibody to determine the actual prevalence of GBV-C/HGV infection. The high prevalence of the GBV-C/HGV markers among the HIV-1-infected subjects, especially those with sexual exposure, provides additional evidence that this route of transmission plays a key role in the epidemiology of GBV-C/HGV. The potential influence of GBV-C/HGV infection on the course of HIV-1 disease needs further evaluation. ***J. Med. Virol. 58:373–377, 1999.***

KEY WORDS: GB virus C; hepatitis G virus; hepatitis C virus; human immunodeficiency virus; sexual transmission; epidemiology

INTRODUCTION

A novel human flavivirus named GB virus C (GBV-C) or hepatitis G virus (HGV) has been identified [Simons et al., 1995; Leary et al., 1996; Linnen et al., 1996]. Although GBV-C/HGV infection had been recognized in some patients with fulminant hepatic failure [Yoshiba et al., 1995; Heringlake et al., 1996; Tameda et al., 1996], no clear relationship between this virus and either acute or chronic liver disease has yet been established. The question of whether GBV-C/HGV is a "human orphan virus" or a true pathogen is still under dispute [Miyakawa and Mayumi, 1997; Theodore and Lemon, 1997; Mushahwar and Zuckerman, 1998]. Epidemiological data show a high prevalence of GBV-C/HGV infection in subjects exposed to parenteral risks, including blood transfusion recipients and hemophiliacs [Schmidt et al., 1996; Alter et al., 1997], intravenous drug users (IVDUs) [Aikawa et al., 1996; Schreirer et al., 1996; Stark et al., 1996], and patients on maintenance hemodialysis [Masuko et al., 1996]. Evidence for vertical [Feucht et al., 1996] and sexual transmission [Stark et al., 1996; Fiordalisi et al., 1997; Kao et al., 1997; Sarrazin et al., 1997; Wu et al., 1997; Ibanez et al., 1998; Scallan et al., 1998] has also been reported.

Sexual transmission has been investigated mainly using the detection of GBV-C/HGV viremia in both homosexual and bisexual males and the heterosexual population, which has demonstrated that GBV-C/HGV

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may spread by sexual contact. However, as GBV-C/HGV has been shown in a high proportion of patients to be eliminated in a period ranging from several months to several years [Lefrère et al., 1997] and also that the antibody response to the glycoprotein E2, a major antigen of the viral envelope [Pilot-Matias et al., 1996], is a specific marker of past GBV-C/HGV infection [Dille et al., 1997; Tacke et al., 1997], the detection of both these markers is necessary to estimate the overall prevalence of GBV-C/HGV infection for epidemiological purposes [Gutierrez et al., 1997; Nübling et al., 1997; Lampe et al., 1998; Scallan et al., 1998].

In order to determine the respective importance of parenteral and sexual transmission in the epidemiology of GBV-C/HGV, the prevalence of both GBV-C/HGV markers (RNA and E2 antibody) and antibodies to the hepatitis C virus (HCV) in patients with the human immunodeficiency virus type 1 (HIV-1) was studied in relation to their route of HIV-1 contamination.

MATERIALS AND METHODS

Patients and Serum Specimens

HIV-1-infected patients were assigned to four groups according to the transmission route recorded in the DMI2 file, a computer program used in France to store epidemiological data on HIV-1-infected subjects: multiple blood products recipients, including hemophiliacs (group 1), IVDUs (group 2), homosexual men (group 3), and heterosexual individuals (group 4). Retrospective random selection was used to assign 20 patients to each group. The last available serum specimen from each patient was used for the study. The patients ranged in age from 20 to 75 (mean: 40); 59 (73.8%) were men. The specimens were collected between January 1993 and March 1997. All samples were stored at –20°C before testing and were not subjected to multiple freeze–thaw cycles.

Detection of GBV-C/HGV RNA

For the detection of GBV-C/HGV by RT-PCR, a protocol described by Boehringer-Mannheim (Germany) was used according to the instructions provided by the manufacturer. Briefly, viral RNA was extracted from 200 μl of serum and reverse-transcribed. Two independent PCR tests were then carried out with primers specific for the 5′ noncoding region (NCR) of GBV-C/HGV genome and for the NS5a region. After the amplification step, the detection of the digoxigenin-labeled products was carried out using the PCR ELISA DIG Detection Kit (Boehringer-Mannheim). Discrepant results with the two pairs of primers were tested once more; after resolution of discrepancies, all the positive samples were found reactive by both PCR reactions.

Antibody Detection

Antibodies to the GBV-C/HGV E2 antigen were tested by ELISA [Tacke et al., 1997], using the μPlate anti-HGenv test (Boehringer-Mannheim) according to the manufacturer's instructions. Antibodies to HCV were evaluated by two commercially available ELISAs (AXSYM, Abbott Diagnostics, France and anti-HCV Version III, Murex, France).

Statistical Analysis

Statistical comparisons were undertaken using variance analysis (F test) for mean values and the chi-square test for percentages.

RESULTS

The results of the study are summarized in Table I.

The demographic data between the four groups show no significant difference either in the mean age of patients or in the number of AIDS cases. Conversely, a significant difference was observed among the four groups in the mean time separating diagnosis of the HIV-1 infection and the present study ($P < .01$): blood product recipients and homosexual men were found to have longer-standing infections than heterosexual subjects and intravenous drug abusers (Table I). This difference is coherent with the development history of the HIV-1 outbreak among risk groups in France. It disappeared when parenteral exposure (groups 1 and 2) was compared to sexual exposure (groups 3 and 4).

The overall prevalence of GBV-C/HGV markers (viremia and E2 antibody) was 66.3% (53 of the 80 HIV-1-infected subjects) without any statistically significant difference among the four groups (Table I). The comparison of the prevalence of GBV-C/HGV viremia among the four groups showed a slight difference ($P < .01$, Table I); this difference was lost when parenteral exposure was compared to sexual exposure. Similarly, no difference was found in the prevalence of antibodies to the GBV-C/HGV E2 protein among the four groups (Table I). In only four serum specimens (7.6% of GBV-C/HGV infected subjects), one in each group, were the GBV-C/HGV RNA and E2 antibody detected simultaneously. The balance between viremia and E2 antibody response in GBV-C/HGV-infected subjects belonging to the different risk groups is illustrated in Figure 1.

Conversely, there was a strong correlation between the presence of anti-HCV antibodies and the parenteral route of transmission ($P < .001$). The same results were obtained for GBV-C/HGV- and HCV-coinfected subjects: 25 of 40 (31.3%) in subjects with parenteral exposure (groups 1 and 2) versus 2 of 40 (5%) in subjects with sexual exposure (groups 3 and 4) ($P < .001$). Moreover, the distribution of GBV-C/HGV infected subjects was roughly equivalent between the anti-HCV positive and negative individuals: 27 of 39 (69%) and 30 of 41 (75%), respectively, whereas infection with GBV-C/HGV alone was about four times more prevalent in subjects with sexual risk (52.5%) than in those with parenteral exposure (12.5%) ($P < .001$).

DISCUSSION

The first point highlighted by the present study is the need to carry out both RNA and anti-E2 antibody

TABLE I. Characteristics of the Subjects and Prevalence of GBV-C/HGV Markers (RNA and Antibody) and of Antibodies to HCV, According to the Route of Transmission of HIV-1

	Route of transmission of HIV-1				Statistical comparisons among the 4 groups: P*
	Group 1[a]	Group 2[b]	Group 3[c]	Group 4[d]	
Effective	20	20	20	20	—
Sex (no. of males)	13	16	20	10	—
Age (mean ± SD in years)	45.9 ± 16.0	37.4 ± 4.7	34.3 ± 7.0	40.5 ± 11.0	NS
Delay between diagnosis of HIV infection and time of the study (mean ± SD in months)	85 ± 28	32 ± 6	70 ± 13	54 ± 12	<.01
Delay between serum collection and time of the study (mean ± SD in months)	21 ± 0.3	15 ± 0.2	18 ± 0.3	12 ± 0.1	NS
Number of patients with AIDS	10	7	12	4	NS
Positive GBV-C/HGV viremia (no./%)	5/25	11/55	6/30	3/15	<.05
Presence of anti-GBV-C/HGV antibodies (no./%)	11/55	5/25	6/30	10/50	NS
GBV-C/HGV-infected patients (no./%)	15/75	15/75	11/55	12/60	NS
Presence of anti-HCV antibodies (no./%)	14/70	18/90	3/15	4/20	<.001
GBV-C/HGV-HCV coinfected patients (no./%)	11/55	14/70	0/0	2/10	<.001

[a]Multiple blood product recipients and hemophiliacs.
[b]Intravenous drug addicts.
[c]Homosexual subjects.
[d]Heterosexual subjects.
*NS: not significant at the 0.05 level.

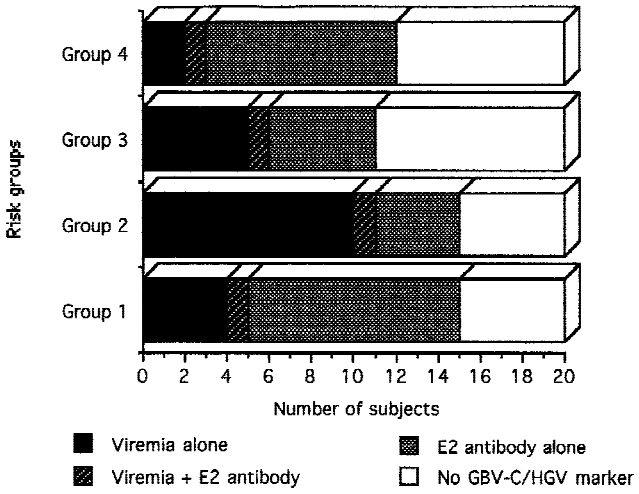

Fig. 1. Distribution of GBV-C/HGV markers according to risk factors of exposure for HIV-1 infection. The risk groups are the same as in Table I.

detection on serum specimens for the correct evaluation of GBV-C/HGV prevalence. As illustrated in Figure 1, if we had been satisfied with detecting only viremia, the overall prevalence of GBV-C/HGV in HIV-1-infected patients, would have been underestimated substantially, especially in those with rapid clearance (as presumed in group 4) or with long-standing infections (group 1). The results also confirm the observations reported in several earlier studies [Pilot-Matias et al., 1996; Dille et al., 1997; Gutierrez et al., 1997; Nübling et al., 1997; Tacke et al., 1997; Lampe et al., 1998] that GBV-C/HGV RNA and E2 antibody are mutually exclusive in most exposed individuals.

One of the principal aims of this study was to provide additional evidence of sexual exposure as an important means of transmission of GBV-C/HGV: no difference was seen in the distribution of patients with markers of GBV-C/HGV infection according to the route of HIV-1 transmission. Of course, as the groups of patients were constituted retrospectively, it is possible that some of the patients experienced more than one mode of HIV-1 exposure (i.e., sexual exposure and use of intravenous drugs). However, the dramatic predominance of HCV seropositivity in groups 1 and 2 (parenteral exposure) as compared to groups 3 and 4 (sexual exposure) suggests strongly that the risk groups were assigned correctly. Various virological and epidemiological findings have pointed to the important role of sexual transmission in the spread of GBV-C/HGV:

1. Detection of GBV-C/HGV RNA in semen [Semprini et al., 1998]
2. High prevalence of GBV-C/HGV RNA [Stark et al., 1996; Fiordalisi et al., 1997; Kao et al., 1997; Sarrazin et al., 1997; Wu et al., 1997; Ibanez et al., 1998] and E2 antibodies [Scallan et al., 1998; this study] in subjects at risk for sexually transmitted diseases (prostitutes, homosexual and bisexual men) or in spouses of GBV-C/HGV-infected subjects
3. Predominant distribution of GBV-C/HGV infection among young sexually active people [Kato et al., 1997; Lampe et al., 1998]
4. High exposure to GBV-C/HGV in a presumably healthy, low-risk population such as blood donors with an overall prevalence (PCR plus antibodies) of 5.5% in the United States [Gutierrez et al., 1997] and 10.4% in Germany (Nübling et al., 1997]

In addition, as already reported by Ibanez et al. [1998], no difference in GBV-C/HGV prevalence was observed between homosexual and heterosexual individuals.

Another aim of this study was to investigate GBV-C/HGV markers in HIV-1-infected patients. When compared to the study of Ibanez et al. [1998], which de-

tected only RNA, the current results show higher prevalences of GBV-C/HGV viremia (25% vs 11.5% in group 1; 55% vs 27% in group 2; 30% vs 19% in group 3 and 15% vs 14% in group 4). The high prevalence of GBV-C/HGV viremia in subjects, many of whom were probably infected many years ago, calls into question another possible effect of the immunosuppression, that of reducing the ability of the immune system to eliminate GBV-C/HGV [Scallan et al., 1998]. For HIV-1-infected homosexual men, the range of GBV-C/HGV viremia prevalence was as follows: 13% [Nübling et al., 1997], 19% [Ibanez et al., 1998], 30% [this study], and 51% [Scallan et al., 1998]. However, these comparisons are not meaningful because the patients were not all at the same stage of GBV-C/HGV infection, and it is therefore more interesting to compare the overall prevalence including RNA plus antibody detection. In HIV-1-infected homosexuals, 32% prevalence was found by Nübling et al. [1997], 55% in this report, and 63% by Scallan et al. [1998].

In conclusion, this study demonstrates the need to combine the detection of viremia and E2 antibody to evaluate the actual prevalence of GBV-C/HGV infection and provides additional evidence for the important role of sexual transmission in the spread of GBV-C/HGV. Although this virus has not yet demonstrated its status as a primary pathogen, its ability to replicate in the human liver has been claimed recently [Mushawar and Zuckerman, 1998].

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REFERENCES

Aikawa T, Sugai Y, Okamoto H. 1996. Hepatitis G infection in drug abusers with chronic hepatitis C. N Engl J Med 334:195–196.

Alter HJ, Nakatsuji Y, Melpolder J, Wages J, Wesley R, Shih JW, Kim JP. 1997. The incidence of transfusion-associated GB virus C/hepatitis G virus infection and its relation to liver disease. N Engl J Med 11:747–754.

Dille BJ, Surowy TK, Gutierrez RA, Coleman PF, Knigge MF, Carrick RJ, Aach RD, Hollinger FB, Stevens CE, Barbosa LH, Nemo GJ, Mosley JW, Dawson GJ, Mushahwar IK. 1997. An ELISA for detection of antibodies to the E2 protein of GB virus C. J Infect Dis 175:458–461.

Feucht H-H, Zöllner B, Polywak S, Laufs R. 1996. Vertical transmission of hepatitis G. Lancet 347:615.

Fiordalisi G, Bettinardi A, Zanella I, Stellini R, Paraninfo G, Cadeo G, Primi D. 1997. Parenteral and sexual transmission of GB virus C and hepatitis C virus among human immunodeficiency virus-positive patients. J Infect Dis 175:1025–1026.

Gutierrez RA, Dawson GJ, Knigge MF, Melvin SL, Heynen CA, Kyrk CR, Young CE, Carrick RJ, Schlauder GG, Surowy TK, Dille BJ, Coleman PF, Thiele DL, Lentino JR, Pachucki C, Mushahwar IK. 1998. Seroprevalence of GB virus C and persistence of RNA and antibody. J Med Virol 53:167–173.

Heringlake S, Osterkamp S, Trautwein C, Tilman HL, Böker K, Muerhoff S, Mushahwar IK, Hunsmann G, Manns MP. 1996. Association between fulminant hepatic failure and a strain of GB virus C. Lancet 348:1626–1629.

Ibanez A, Gimenez-Barcons M, Tajahuerce A, Tural C, Sirera G, Clotet B, Sanchez-Tapias J-M, Rodes J, Martinez M-A, Saiz J-C. 1998. Prevalence and genotypes of GB virus C/hepatitis G virus (GBV-C/HGV) and hepatitis C virus among patients infected with human immunodeficiency virus: Evidence of GBV-C/HGV sexual transmission. J Med Virol 55:293–299.

Kao J-H, Liu C-J, Chen P-J, Chen W, Hsiang S-C, Lai M-Y, Chen D-S. 1997. Interspousal transmission of GB virus-C/hepatitis G virus: a comparison with hepatitis C virus. J Med Virol 53:348–353.

Kato T, Mizokami M, Nakano T, Kondo Y, Ohba K-I, Orito E, Ueda R, Mukaide M, Gurtsevitch V, Syrtsev A, Ruzibakiev R, Abdurakhnamov M, Yamashita M, Hayami M. 1997. High prevalence of GB virus C/hepatitis G virus infection among the Jewish population in Uzbekistan. Virus Res 48:81–87.

Lampe E, Saback FL, Viazov S, Roggendorf M, Niel C. 1998. Age-specific prevalence and genetic diversity of GBV-C/hepatitis G virus in Brazil. J Med Virol 56:39–43.

Leary TP, Muerhoff AS, Simons JN, Pilot-Matias TJ, Erker JC, Chalmers ML, Schlauder GG, Dawson GJ, Desai SM, Mushahwar IK. 1996. Sequence and genomic organization of GBV-C: A novel member of the Flaviviridae associated with human non A-E hepatitis. J Med Virol 48:60–67.

Lefrère JJ, Loiseau P, Maury J, Lasserre J, Mariotti N, Ravera N, Lerable J, Lefèvre G, Morand-Joubert T, Girot R. 1997. Natural history of GBV-C/hepatitis G virus infection through the follow-up of GBV-C/hepatitis G virus-infected blood donors and recipients studied by RNA polymerase chain reaction and anti-E2 serology. Blood 90:2776–2780.

Linnen J, Wages J, Zhang-Keck Z-Y, Fry KE, Krawczynski KZ, Alter H, Koonin E, Gallagher M, Alter M, Hadziyannis S, Karayiannis P, Fung K, Nakatsuji Y, Shih JW-K, Young L, Piatak JM, Hoover C, Fernandez J, Chen S, Zou J-C, Morris T, Hyams KC, Ismay S, Lifson JD, Hess G, Foung SKH, Bradley TH, Margolis H, Kim JP. 1996. Molecular cloning and disease association of hepatitis G virus: a transfusion-transmissible agent. Science 271:505–509.

Masuko K, Mitsui T, Iwano K, Yamazaki C, Okuda K, Meguro T, Murayama N, Inoue T, Tsuda F, Okamoto H, Miyakawa Y, Mayumi M. 1996. Infection with hepatitis GB virus C in patients on maintenance hemodialysis. N Engl J Med 334:1485–1490.

Miyakawa Y, Mayumi M. 1997. Hepatitis G virus. A true hepatitis virus or an accidental tourist? N Engl J Med 336:795–796.

Mushahwar IK, Zuckerman JN. 1998. Clinical implications of GB virus C. J Med Virol 56:1–3.

Nübling CM, Bialleck H, Fürsch AJ, Scharrer I, Schramm W, Seifried E, Schmidt U, Staszewski S, Löwer J. 1997. Frequencies of GB virus C/hepatitis G virus genomes and of specific antibodies in German risk and non-risk populations. J Med Virol 53:218–224.

Pilot-Matias TJ, Carrick RJ, Coleman PF, Leary TP, Surowy TK, Simons JN, Muerhoff AS, Buijk SL, Chalmers ML, Dawson GJ, Desai SM, Mushahwar IK. 1996. Expression of the GB virus C E2 glycoprotein using the Semliki Forest virus vector system and its utility as a serologic marker. Virology 225:282–292.

Sarrazin C, Roth WK, Zeuzem S. 1997. Heterosexual transmission of GB virus-C/hepatitis G virus infection. Eur J Gastroenterol Hepatol 9:1117–1120.

Scallan MF, Clutterbuck D, Jarvis LM, Scott GR, Simmonds P. 1998. Sexual transmission of GB virus C/hepatitis G. virus. J Med Virol 55:203–208.

Scheirer E, Höhne M, Künkel U, Berg T, Hopf U. 1996. Hepatitis GBV-C sequences in patients infected with HCV contaminated anti-D immunoglobulin and among i.v. drug users in Germany. J Hepatol 25:385–389.

Schmidt B, Korn K, Fleckenstein B. 1996. Molecular evidence for transmission of GB virus C/hepatitis G virus by blood transfusion. Lancet 347:909.

Semprini AE, Persico T, Thiers V, Oneta M, Tuveri R, Serafini P, Boschini A, Giuntelli S, Pardi G, Bréchot C. 1998. Absence of hepatitis C virus and detection of hepatitis G virus/GB virus C RNA sequences in the semen of infected men. J Infect Dis 177:848–854.

Simons JN, Pilot-Matias TJ, Leary TP, Dawson GJ, Desai SM, Schlauder GG, Muerhoff AS, Erker JC, Buijk SL, Chalmers ML, Van Sant CL, Mushahwar IK. 1995. Identification of two flavivirus-like genomes in the GB hepatitis agent. Proc Nat Acad Sci USA 92:3401–3405.

Stark K, Bienzle U, Hess G, Engel AM, Hegenscheid B, Schlüter V. 1996. Detection of the hepatitis G virus genome among injecting

drug users, homosexual and bisexual men, and blood donors. J Infect Dis 174:1320–1323.

Tacke M, Kiyosawa K, Stark K, Schlueter V, Ofenlech-Haehnle B, Hess G, Engel AM. 1997. Detection of antibodies to a putative GB virus C/hepatitis G virus envelope protein. Lancet 349:318–320.

Tameda Y, Kosaka Y, Tagawa S, Takase K, Sawada N, Nakao H, Tsuda F, Tanaka T, Okamoto H, Miyakawa Y, Mayumi M. 1996. Infection with GB virus C (GBV-C) in patients with fulminant hepatitis. J Hepatol 25:842–847.

Theodore D, Lemon SM. 1997. GB virus C, hepatitis G virus, or human orphan flavivirus? Hepatology 25:1285–1286.

Yoshiba M, Okamoto H, Mishiro S. 1995. Detection of the GBV-C hepatitis virus genome in serum from patients with fulminant hepatitis of unknown etiology. Lancet 346:1131–1132.

Wu J-C, Sheng W-Y, Huang Y-H, Hwang S-J, Lee S-D. 1997. Prevalence and risk factor analysis of GBV-C/HGV infection in prostitutes. J Med Virol 52:83–85.